AMMONIA INHIBITION OF THE GENERAL AMINO ACID PERMEASE AND ITS SUPPRESSION IN NADPH-SPECIFIC GLUTAMATE DEHYDROGENASELESS MUTANTS OF SACCHAROMYCES CEREVISIAE

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SUMMARY

Evidence is presented showing that the NADPH-linked glutamic dehydrogenase (anabolic GDHase) plays a key role in the regulation of nitrogen catabolism.

In a mutant lacking anabolic GDHase activity (gdh-A mutation) the sensitivity of the general amino acid permease to ammonia is strongly depressed and three other independent activities are also largely liberated from ammonia inhibition.

Neither intracellular ammonia nor 2-oxoglutarate alone can be considered as effectors of this regulation, but NADPH, NADP, the enzyme itself, or combinations of different effectors were not eliminated.

The physiological function of the general amino acid permease (which exists in addition to a number of specific amino acid uptake systems in Saccharomyces cerevisiae) most probably is to provide the cells with amino acids as nitrogen sources (1). As shown here, the regulation of this uptake system appears to be essentially adapted to this function, since it seems to be integrated in a general regulation of nitrogen catabolism.

It was shown previously that the uptake rate of a number of amino acids is greater in cells grown with proline (rather than ammonia) as a source of nitrogen. This enhancement is due to the activity of a general amino acid permease. This activity is lost as a result of the gap mutation (1). It was apparent from the beginning that, in proline-grown cells in which the general amino acid permease is fully active, the inhibition of the general amino acid permease by ammonia was not of a simple competitive type as that observed with amino acids. The inhibi-

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tion at the time zero of addition of ammonia never reaches 100 % (whatever the concentration of ammonia or of the labelled amino acid used to measure the general amino acid permease activity). In addition, this time-zero inhibition is not maximal : inhibition increases with time and reaches 100 % after about 1 hour only (1).

The purpose of the present paper is to analyze the mechanism of this inhibition.

MATERIALS AND METHODS

Medium and growth conditions were previously described (2). Medium 149 supplemented with 3 % glucose and vitamins was used throughout. Minimal ammonium medium (M. am) contained 0.02 NH_4^+ (as ammonium sulfate) as the sole source of nitrogen ; minimal proline medium (M. pro) and minimal glutamate medium (M. glt) are identical to M.am, except that ammonia is replaced by L-proline and L-glutamate (1 mg/ml) respectively. M.pro+am contains ammonia and L-proline simultaneously, while M. glt+am contains ammonia and L-glutamate.

The uptake rate was measured as previously (2) : after introducing a (^{14}C)-labelled substrate into a culture growing exponentially, samples were removed every half minute for 2 minutes, filtered, washed with ice-cold distilled water, and assayed for radioactivity.

Estimation of the internal concentration of ammonia and 2-oxo-glutarate. After extraction of the acid soluble pool in ice-cold 0.3 M $HClO_4$ and neutralization by K_3PO_4, ammonia or 2-oxo-glutarate were assayed with beef liver glutamate dehydrogenase (Boehringer). The detailed procedure, as well as the assay of glutamate dehydrogenase activity in extracts of yeast cultures will be described elsewhere (Dubois, Wiame and Grenson, in preparation).

Genetic analysis was performed as previously (2).

RESULTS AND DISCUSSION

1. Isolation of mutants in which the general amino acid permease is resistant to ammonia inhibition :

Concentrations of arginine (such as 1.5×10^{-4}M), which allow optimal growth of single arginine-less (arg^-) strains, only permit very slow growth of double arginine-less, arginine-permease-less mutants (arg^-, arg-p) in M.am (2), M.pro+am or M.glt+am medium.

However, in M.pro medium, normal growth is restored on the same arginine concentrations, because arginine enters the cells through the general amino acid permease under these conditions.

From the strain 2727a (arg^-, arg-p), mutants were isolated which are able to grow normally on M.glt+am or M.pro+am medium supplemented with 1.5×10^{-4} M L-arginine. Genetical analysis showed that they belong to several complementation classes, one of which will be described here. To eliminate the arg^- and the arg-p mutations, each mutant was crossed with a wild-type strain of opposite mating type, and a haploid segregant bearing the new mutation only was selected. Strain 4324c is such a segregant, recovered from the mutant MG841.

The growth of strain 4324c on M.am medium is slow (the mean generation time is about 3 h 45 instead of 2 h), but it is normal on other sources of nitrogen like glutamate, aspartate, proline, arginine, valine, methionine, tryptophan or threonine. This could be due either to a lowered rate of ammonia uptake, or to a defect in ammonia metabolism.

2. Ammonia accumulation.

The internal concentration of exogenous ammonia in the mutant cells is about 6 fold higher than in the wild-type strain grown under the same conditions (Table I), which shows that the uptake of ammonia is not limiting.

3. Glutamate dehydrogenase activity.

It is known that the yeast Saccharomyces cerevisiae possesses two glutamate dehydrogenases, one of which is responsible for the synthesis of glutamic acid from ammonia, 2-oxo-glutarate and NADPH (anabolic GDHase); the other one (catabolic GDHase) is specialized in the liberation of ammonia from glutamic acid (as indicated by its twenty fold repression by ammonia) and requires NAD as a cofactor (3, 4).

The activity of the NADPH-linked glutamate dehydrogenase is absent in strain 4324c, whereas the NAD-linked glutamate dehydrogenase activity is present. Hence, the mutation has been named gdh-A.

4. Activity of the general amino acid permease in the presence of ammonia.

The inhibition of amino acid uptake after growth in the presence of ammonia is strongly reduced in the gdh-A mutant (Table II).
To measure the activity of the general amino acid permease without interferences with other uptake systems, uptake was measured in cells

TABLE I

Internal concentration of ammonia in the wild-type strain and in the mutant strain 4324c

Strains	nanomoles ammonia/mg dry weight of cells grown in		
	M.am	M.pro	M.pro+am
Σ1278b (w.t.)	18	4	25
4324c (mutant)	115	5	150

TABLE II

Amino acid uptake

Substrate (0.1 mM)	Initial uptake rate (as nanomoles/mg protein/min) in "wild-type"strains		gdh-A mutants		gdh-A, gap
	M.pro*	M.pro+am*	M.pro*	M.pro+am*	M.pro+am*
L-tryptophan	79(a)	0.7(a)	84(b)	69(b)	1.25(c)
L-citrulline	46(a)	0.1(a)	57(b)	35(b)	1.0 (c)
L-arginine	77(d)	2.4(d)	68(e)	47(e)	-
L-lysine	74(f)	5.0(f)	80(g)	46(g)	-

* culture medium
particular strains used : (a) Σ1278, w.t. ; (b) 4324c, gdh-A ; (c) 10247a, gdh-A, gap ; (d) MG168 (arg-p) ; (e) 4325d, gdh-A, arg-p ; (f) RA382, lys-p ; (g)4410a (gdh-A, lys-p).

bearing additional mutations which inactivate the specific arginine (arg-p) and lysine (lys-p) permeases respectively. In the case of tryptophan and citrulline, the uptake by other permeases may be considered as negligible in regard to the activity of the general amino acid permease (1). That the reduction of sensitivity to ammonia concerns the activity of the general amino acid permease is confirmed

by the fact that a double mutant (gdh-A, gap), strain 10247a, grown on M.pro+am medium, does not show the high uptake rate of amino acids which is observed in a single gdh-A mutant (Table II).

5. Why does a gdh-A mutation lead to decreased sensitivity of the general amino acid permease to ammonia ?

A simple explanation for the fact that the general amino acid permease is active in the gdh-A mutant grown in M.am medium would be that, due to a lowered pool of endogenous amino acids, the general amino acid permease is liberated from feedback inhibition. That the pool of amino acids is low in strain 4324c grown on M.am medium is not only suggested by slow growth rate, but is confirmed by amino acid analysis. However, when proline is added to the M.am medium, the loss of activity of the GDHase A in the mutant is completely compensated from the point of view of glutamate formation : the internal concentration of glutamate and the pool of amino acids are practically identical to that of a wild-type strain (to be published elsewhere). Nevertheless, resistance of the general amino acid permease to ammonia inhibition is still observed under these conditions (Table II).

When trying to understand why the gdh-A mutation leads to ammonia resistance, it is tempting to suppose that one of the other consequences of the absence of this enzyme activity is responsible for this effect. In other words, it might be asked wether 2-oxo-glutarate or NADPH can act as activators of the general amino acid permease or, conversely, whether NADP is an inhibitor. From a physiological point of view, this would be a satisfactory situation, since these substances would be good signals of the availability of a nitrogen source.

6. Internal concentration of 2-oxo-glutarate.

The results in Table III show that 2-oxo-glutarate is not the activator (or is not the sole effector) of the system, since its level is low in the gdh-A mutant grown in M.am or in M.pro+am medium, whereas the activity of the general amino acid permease is high under these conditions (see Table II, f.i.).

7. Other effects of the gdh-A mutation.

Two uptake systems distinct from the general amino acid permease, the proline uptake system (1, 5) and the ureidosuccinate uptake system

TABLE III

Internal concentration of 2-oxo-glutarate*

	Σ1278b (w.t.)	4324c (gdh-A)
M.am	0.7 ± 0.1	0.6 ± 0.1
M.pro	5.4 ± 0.2	4.6 ± 0.1
M.pro+am	0.7 ± 0.1	0.8 ± 0.1
M.glt	4.5 ± 0.2	6.6 ± 0.2
M.glt+am	0.7 ± 0.1	1.9 ± 0.2

* in nanomoles/mg dry weight.

TABLE IV

Effect of the gdh-A mutation on the activity of three permeases in the presence of ammonia.

Strains	Cells grown in M.am medium			Cells grown in M.pro+am medium		
	g	v_i uptake		g	v_i uptake	
		proline (0.1 mM)	ureido-succinate (0.2 mM)		tryptophan (0.02 mM)	ureido-succinate (0.2 mM)
Σ1278b (w.t.)	2h	0.1	<0.1	2h	<0.1	<0.1
4324c (gdh-A)	3h50	10.3	28.9	2h	22.1	23.3

g = mean generation time
v_i = initial velocity of uptake in nanomoles/min/mg protein.

(6) are known to be inhibited in cells grown in the presence of ammonia. The gdh-A mutation also suppresses the inhibitory effect of ammonia on these uptake systems (Table IV).

The central role of the NADPH-linked GDHase in the regulation of

nitrogen catabolism in yeast becomes clearly apparent in the following observation. The synthesis of arginase, the first enzyme of arginine catabolism, is known to be regulated by induction (by arginine) and repression (by ammonia) (see 7). The repression by ammonia (catabolic repression) is also suppressed by the gdh-A mutation (Dubois, Wiame and Grenson, to be published elsewhere).

CONCLUSION

Although the reason why the gdh-A mutation suppresses or reduces ammonia inhibition or catabolic repression on several systems is not yet clearly understood, it may be concluded that the NADPH-linked GDHase plays a key role in the regulation of nitrogen catabolism (defined as the metabolic processes transforming nitrogen sources into molecules which can be used as building blocks). The possibilities which must be investigated on the basis of the results presented here are the following. Firstly, regulation might operate through NADPH or NAPH. Secondly, an intervention of the enzyme molecule itself may not be overlooked. Finally, it is possible that regulation is the result of the action of several effectors acting either in a cooperative or in an antagonistic way.

Mutations which pleiotropically suppress the inhibitory effect of ammonia on uptake systems and/or enzymes involved in nitrogen metabolism have been described in yeast (6) and in Aspergillus nidulans (8, 9). On the basis of the available data, these mutations do not seem to inactivate glutamate dehydrogenase, and some (9) are probably affected in ammonia transport. However, some of the mutations allowing ureidosuccinate utilization in the presence of ammonia lead to reduced levels of NADP-GDHase activity in yeast (Drillien and Lacroute, personnal communication).

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